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13. ABSTRACT *(Maximum 200 Words)*

Bleeding from an artery is difficult to control due to the high pressures found in the arterial system. Hemorrhage is especially problematic in penetrating wounds where the bleeding source may not be apparent. Tourniquets that are routinely used to treat such wounds can cause multiple complications. We are developing a device which, when exposed to aqueous solutions, rapidly generates pressure in a confined space. In this report, we summarize the design and testing of a prototype device. The "biohemostat" is composed of a flexible outer membrane, which surrounds a hydrophilic, super-absorbent polymer. The outer bag is made from an electrospun mat of Ethylene-vinyl acetate co-polymer. The electrospun mat is very flexible, durable (stretching to 10 times its original length), biocompatible and porous. Its relative degree of hydrophobisity is overcome by incorporating a percentage of EVOH either as a blend or composite. The hydrophilic polymer used in the prototype device is composed of polyacrylic acid derivatives or copolymers. When the device is placed in aqueous solutions it rapidly absorbs fluid, expands and develops significant pressure in a confined space. Although swelling of such polymers is dependent on the nature of the aqueous solution (i.e. Varies with pH, ionic strength, protein content, etc.) the decreases in absorption caused by these parameters have been easily overcome by increasing the amount of hydrophilic polymer. The goal is to develop a device, which can be placed directly into a wound to develop counter pressure to aid in hemorrhage control. By developing pressure directly on the bleeding site, the hope is to avoid the crush injuries and ischemic damage associated with tourniquet use.

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C. Table of Contents

D. INTRODUCTION

Ballistic injury is a primary mode of trauma in combat. Such injuries can be associated with rapid blood loss due to vascular disruption. In the Vietnam conflict, ten percent of wounds to the extremity were associated with major artery injury.[1] While bleeding from compressible vessels may respond to direct pressure, blood loss from deep muscular branches such as those from the profunda femoris artery may be severe.[2] Despite increasingly aggressive surgical treatment, limb salvage has not improved[3], and death from hemorrhagic shock remains a problem even in very healthy individuals.[4] Combat vascular injuries continue to result in a 12 to 30 percent amputation rate depending on the involved vessel.[5]

Described more than 2000 years ago as an adjuvant to surgical amputation[6], tourniquets have become a primary initial treatment of injuries with associated high pressure bleeding. Unfortunately, tourniquet utilization can be associated with a variety of complications including nerve injuries, distal ischemia, compartment syndromes, post-tourniquet syndrome, and pulmonary embolus.[7,8] A major consequence of these complications is an increased risk of limb wastage. Despite these potential complications, combat as recent as the 1991-92 Croatian conflict has verified the ability of tourniquets to delay shock in lower extremity arterial injuries.[9]

Recent developments in the field of hemostatic agents have raised the possibility of alternative treatment of vascular bleeding. The development of virally inactivated fibrin sealant and its documentation as a useful adjuvant to multiple types of surgery have been major advances.[10,11] The effectiveness of fibrin glue in speeding hemostasis along vascular graft suture lines[12], presaged its testing as an adjuvant to surgery in the treatment of complex hepatic injury.[13] Alternate formulations of fibrinogen and thrombin containing dressings[14] and dry fibrin sealant dressings[15] have prompted studies of these dressings in pig models of vascular injury[14] and grade V liver injury.[15,16] Dry fibrin sealant dressing was recently shown to be more effective than standard gauze in decreasing bleeding and maintaining blood pressure in ballistic injury.[17]

While the development of "dry" products has increased their potential as alternatives to tourniquets for battlefield treatment, several potential problems remain. First, these products are very expensive. Second, although virally inactivated, the fibrinogen they contain comes from multiple human donors and cannot be considered totally safe in terms of pathogen transmission. Third, these products must be held in place until bleeding stops or the material may simply wash out of the wound. This is especially true when the bleeding is brisk as with arterial involvement. The need for a tourniquet alternative that is effective, inexpensive, lacks viral risk, and can be easily administered by an army medic is obvious.

Superabsorbent polymers are crosslinked hydrophilic polymer networks with the ability to absorb large quantities of pure water, saline or physiological solutions.[18,19] Superabsorbent polymers can absorb large amounts of water or other fluids and swell up to thousands of times their own weight in aqueous media. The absorbed water is retained within the network even under considerable pressure.[20,21] Superabsobent polymers have been utilized in a variety of applications including drug delivery systems, absorption pads, consumer care products, disposable diapers, hygienic napkins, biomedical materials, soft contact lenses, supports for catalysts, soil components for agriculture and horticulture, gel actuators, water blocking tapes, and artificial snow for winter sports.[22-25]

The electrostatic spinning (electrospinning) process is an attractive approach for processing polymer biomaterials because it offers the opportunity for control over material morphology, porosity, and composition using simple equipment. In electrospinning, polymer solutions or melts are deposited as fibrous mats rather than droplets. At sufficiently high polymer concentrations, chain entanglements in melts allow production of continuous fibers. The fibers are produced by charging the liquid to 5000-30,000 Volts vs. a ground a short distance away. This leads to injection of the charged liquid from the catheter type electrode and capture of the forming polymer on a device placed between the catheter and the electrical ground.

Electrospinning is a cost effective method for producing fibrous polymer mats with fiber diameters ranging from 0.01 μm to several tens of μm.[26-29] Such materials may be useful for many applications in medicine such as wound dressings and scaffolds for tissue engineering.[30-32] The simplicity of the electrospinning process itself, the ability to control the fiber diameter and overall porosity of the resulting mat, and the ability to incorporate therapeutic compounds into the mats during spinning, afforded the prospect of preparing useful polymer systems for controlled drug delivery. While flat mats represent an attractive form for topical delivery applications, other shapes (e.g., tubes) can be can be constructed using different target geometries.

We used the electrospinning technique to manufacture the outer (highly permeable) bag of the biohemostat device. We had previously shown the utility of this approach in the delivery of drugs such as the antibiotic tetracycline Hydrochloride.[33]
Due to it's well know biocompatibility, ethylene-vinyl acetate copolymer was selected for this biohemostat applications.[34-36] EVA has been used in many biomedical applications including controlled release of drugs and macromolecules such as immunoglobulin G.[37,38] In some applications, such as controlled release of insulin, EVA has been used as an implantable polymer.[39] Its use in the field of dental diseases has also been reported.[40,41]

In this report, we detail work accomplished during the first year of a grant (DAMD 17-01-1-0691) from the United States Army. Progress toward the design and construction of a new hemostatic device composed of a porous outer bag containing superabsorbent polymers is summarized.

E. BODY

Work Accomplished will be detailed by the specific goals listed in our approved statement of work.

The original device concept involved a relatively small, flexible bandage that would be placed directly in the wound. Once in place, liquid within the wound would rapidly penetrate the bandage and be absorbed by super-absorbent polymers contained within the bandage. As a consequence of fluid absorption, the bandage would expand and develop pressure within the wound to aid in hemorrhage control.

Development of the Outer (encasement membrane) Material(Goals 6 and 7)

Based on this simple design, the outer material of the device had to meet certain functional criteria (Goals 6 and 7). The outer coating must be: flexible, porous, biocompatible, wettable, and durable. We chose electrospinning (Figure 1 and 2) as the process by which we would prepare various sheets of candidate polymers for initial testing. Electrospinning is simple, inexpensive and

allows the production of polymer sheets of varying size, thickness and shape. Ethylene Vinyl Acetate (EVA) was initially chosen as a candidate material due to its high flexibility (figure 3), large pore size (see figure 4) and biocompatibility. As can be seen by scanning electron microscopy (figure 5), electrospun EVA is composed of a continuous string of polymer (i.e. there are no obvious polymer endpoints). This property increases structural integrity and therefore decreases the possibility of device rupture during use or removal.

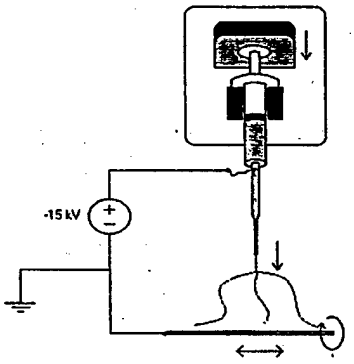


Figure 1. Schematic of Electrospinning

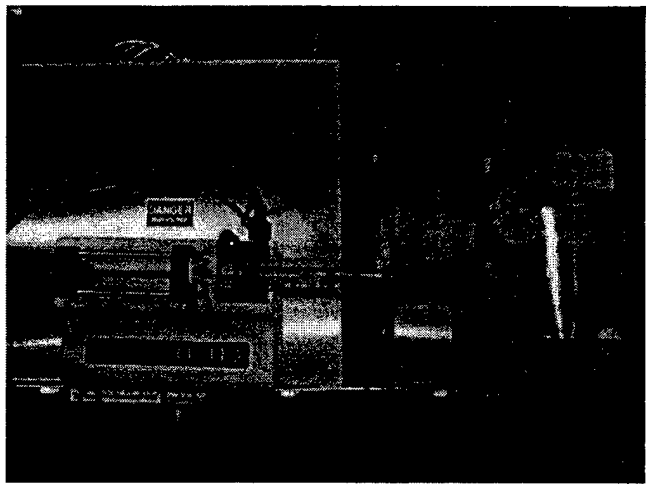


Figure 2.

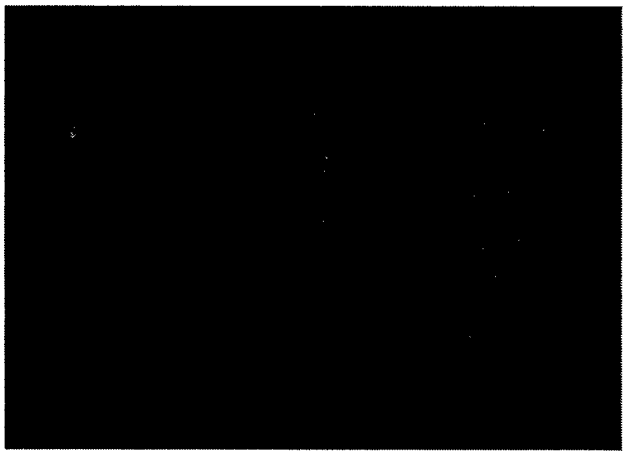


Figure 3.

Figure 4.

Unfortunately, EVA is not extremely wettable. This property slowed initial swelling of prototype devices (Figure 6). Once swelling began, it proceeded rapidly due to the opening of large pores in the EVA network. The period prior to rapid swelling was too excessive to allow EVA to function as the outer device material.

Addition of hydroxyl (OH) groups to EVA produces Ethylene Vinyl Alcohol (EVOH), which is dramatically more wettable but much less flexible (Figure 7). By combining alternating

layers of EVA and EVOH a material was produced with the requisite outer-shell properties (see figure 8).

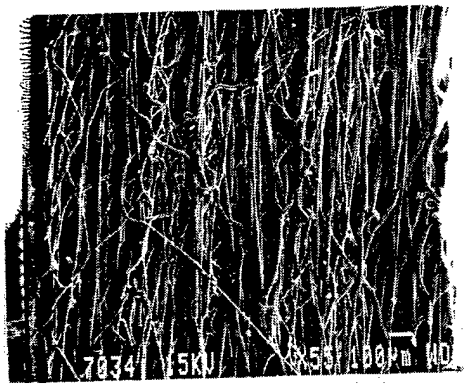


Figure 5.

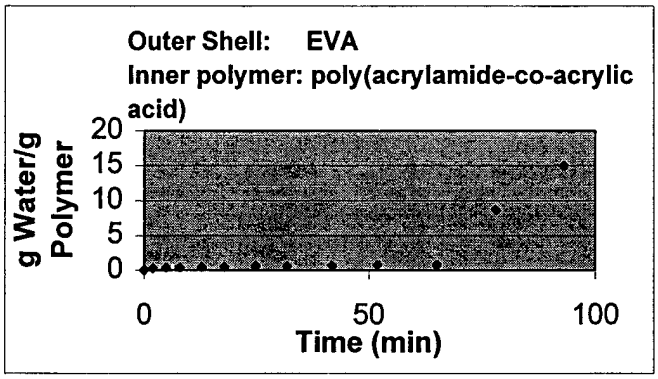


Figure 6.

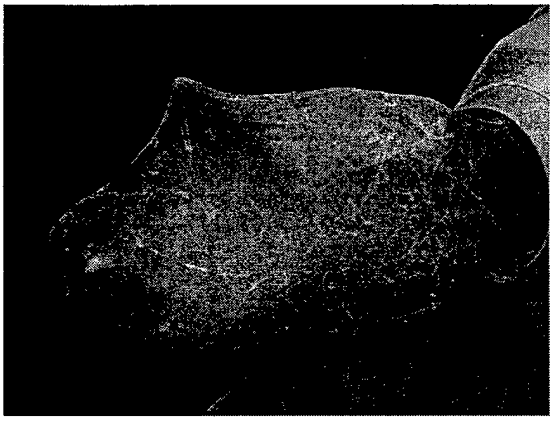


Figure 7.

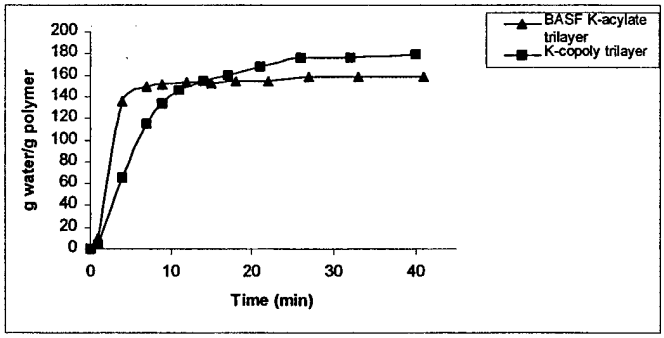


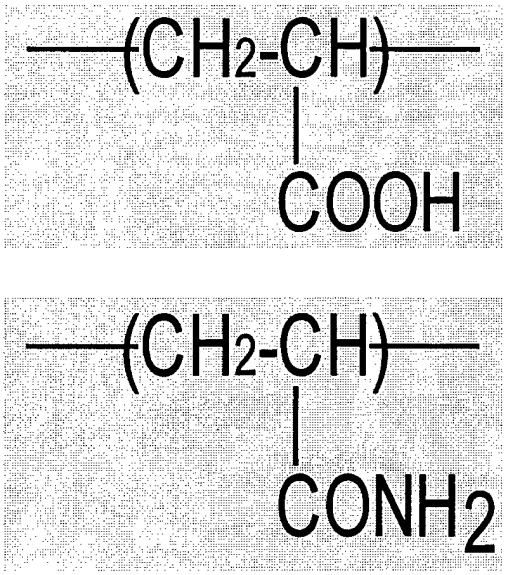
Figure 8.

Selection and optimization of Super-Absorbent Inner Polymer Materials (Goals 1 and 2)

Testing of several candidate commercially available super-absorbent polymers identified poly(acrylic-co-acrylamide) (Figure 9) as an appropriate hydrophilic polymer for the BioHemostat device. The speed of swelling was optimized by the addition of potassium salt (Figure 10, compare to figure 6) and by polymer neutralization (figure 11). Direct comparison of the neutralized polymer and the potassium salt (Figure 12) lead to the acceptance of the Poly(acrylic-co-acrylamide) potassium salt as the best candidate for further device development (figure 13). This polymer rapidly expands, absorbing up to 1,000 times its weight in water.

The absorption characteristics of all super-absorbent polymers are to some degree dependent on the nature of the aqueous environment. That is to say, absorption is altered by increasing ionic strength, changes in pH and the presence of proteins. Since the BioHemostat will be utilized at an

ionic strength of 0.15M, a pH of 7.4 and in the presence of large amounts of protein, the ability of prototype devices to absorb salt solutions, plasma and blood were therefore studied. As anticipated, increased ionic strength and the presence of protein slowed and decreased absorption. This was most easily overcome by simply increasing the amount of absorbent polymer contained within the devices (Figure 14). Subsequent testing in whole blood (figures 15 and 16) confirmed the ability of the device to absorb in this environment.



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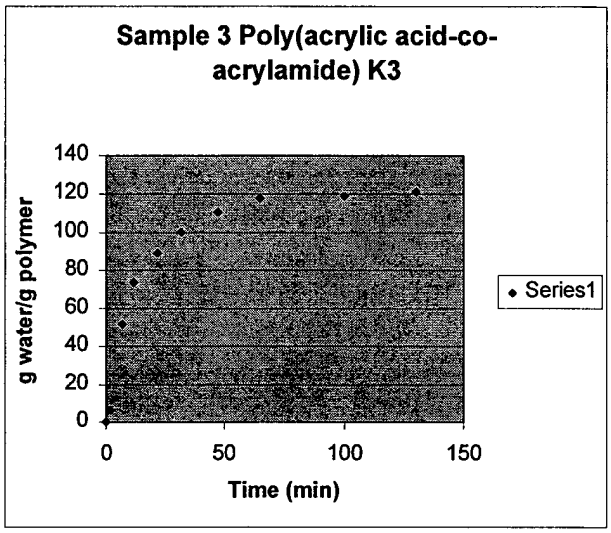

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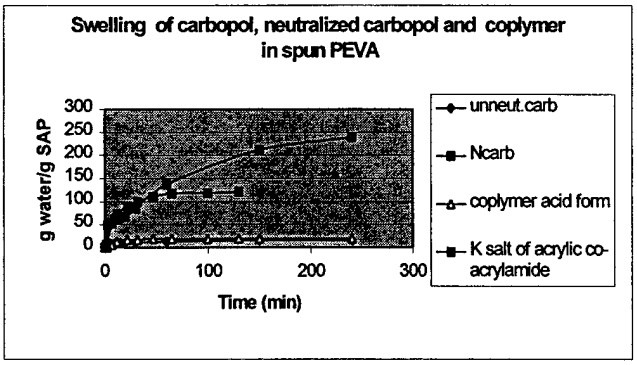

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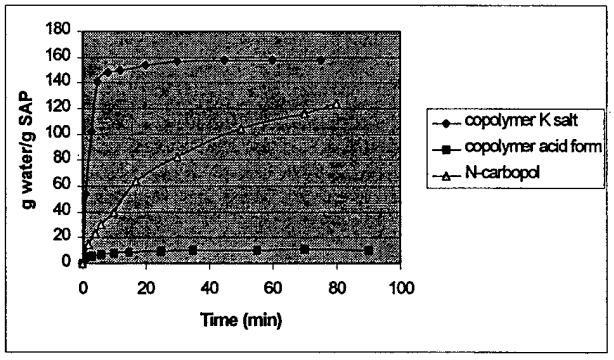

Figure 12.

Figure 13.

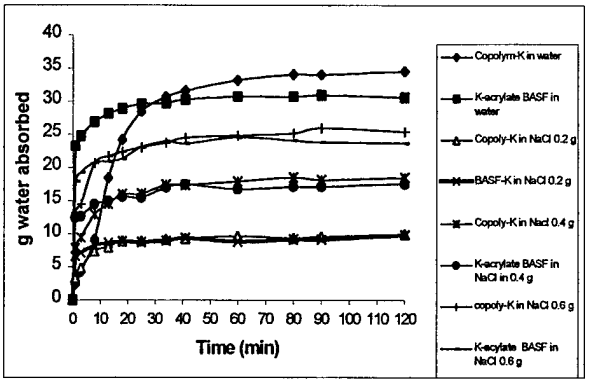

Figure 14.

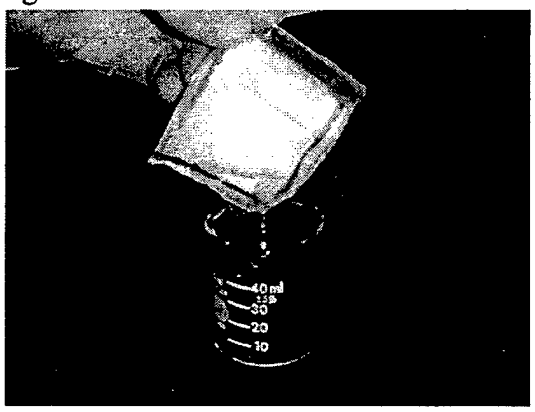

Figure 15.

Figure 16.

Documentation of the ability of the device to develop force in a confined space (Goals 3 and 5)

Testing of prototype devices composed of outer EVA-EVOH composite shells containing Poly(acrylic-co-acrylamide) potassium salt has recent verified their capacity for force generation in a simple confined space model (figures 17-19). Additional studies are ongoing to optimize this ability as animal trials are approached.

Figure 17.

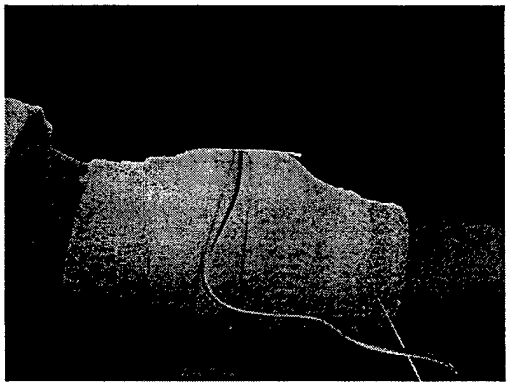

Figure 18.

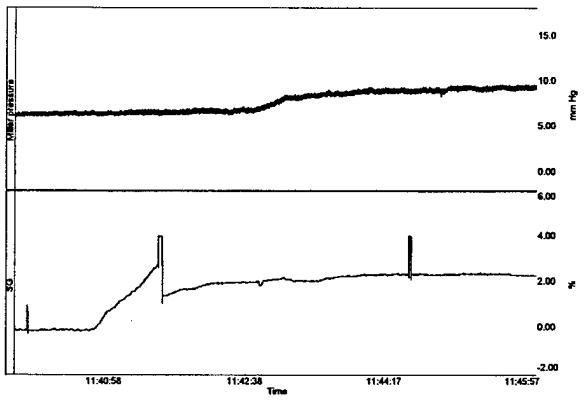

Figure 19.

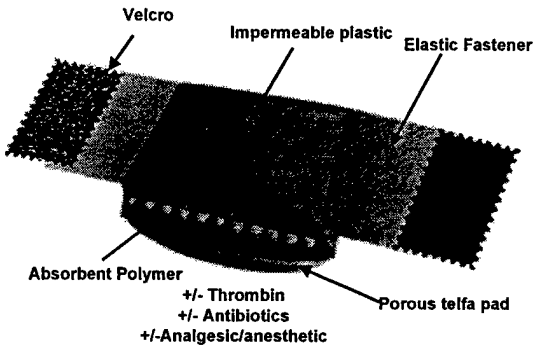

Figure 20.

Addition of a hemostatic agent to the device to speed clot formation (Goals 4 and 8)

Thrombin is the only agent tested to this point. Attempts have been made to incorporate thrombin in the EVA-EVOH mat during electrospinning. These have been minimally successful to this point. A minimal shortening (120 to 110 seconds) of the clotting time was noted when 5.6 NIH units of thrombin were electrospun into a 200 cm^2 EVA-EVOH mat. We are convinced that this simply represents the use of too little thrombin. Ongoing experiments will incorporate several hundred units of thrombin on a similar piece of material.

Initiate testing of the prototype device testing in a ballistic animal model (Goals 10 and 11)

Although our animal model is approved by the university IACUC and safety committees, we have been unable to initiate these studies for several reasons. Concerns have been raised about physical security at the VCU Sanger Hall Facility given the frequent appearances of animal rights activists, demonstrators and protestors at that location. Therefore, even though approved, the use of a large animal, ballistic injury model may prove problematic at that location. We are looking at the possibility of moving this portion of the study to the Richmond VA Medical Center Animal studies facility. Conversations have been ongoing, and the McGuire Research Institute of the Richmond VA is willing to act as a "vendor" for this part of the study. Problems initially arose over the possibility of potential intellectual property claims by the VA health care system. It now appears that these can be circumvented by the "vendor" relationship. A second problem has been the unanticipated expense of the bullet trap ($38,000) and the necessary modifications of the facility to accommodate this equipment. We are working with the University administration to see if some indirect funds can be used to defray some of this expense. We have also been approached by COL Tom Reid of Walter Reed Army Institute of Research with the option of using one of their three bleeding animal models in the testing of our prototype devices. We may have to consider shifting our animal studies from a ballistic to a surgically induced vascular injury model, if some of these difficulties can not be resolved.

F. KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated the utility of electrospinning in the "tailoring" of candidate polymers
- Tested the structural, chemical and performance characteristics of a variety of candidate

polymers for the device outer shell
- Selected the outer polymer for the device
- Optimized the properties of the outer shell via a composite of two polymers
- Tested a variety of potential hydrophilic, super absorbent polymers
- Defined critical parameters for rapid swelling
- Selected the inner hydrophilic, hyper-absorbent polymer
- Optimized the properties of the inner polymer in terms of salt content and neutralization
- Constructed a variety of prototype devices
- Demonstrated the rapid swelling and the potential for force development in water
- Defined the effects of increasing ionic strength, pH and plasma proteins on device performance
- Demonstrated the feasibility of adding a hemostatic agent to the device
- Proposed additional versions of the device which envision and will allow:
    - Rapid (within a few seconds) pneumatic force development
    - Incorporation of analgesics in the device
    - Incorporation of antibiotics in the device
    - Incorporation of other hemostatic agents in the device

G. REPORTABLE OUTCOMES

Manuscripts (one):

Kenawy ER, Carr ME, Wnek G. Development of the Bio-Hemostat – A Treatment Modality for High Pressure Bleeding Based on Super Absorbent Polymer and Electrospun Bag. Manuscript in Preparation.

Abstracts (two):

Carr M, Wnek G, Cohen K, Ward K, Ivatory R, Bowlin G. The BioHemostat – Acute treatment modality for High Pressure Bleeding – Changing the Treatment Paradigm to: "Saving the Patient and Sparing the Limb." Proceedings of ATACC 2001 – Advanced Technology Applications for Combat Casualty Care. Page 30, Abstract 44.

Carr M, Kenawy E, Layman J, Wnek G, Ward K, Barbee W, Tiba M. "Development of the BioHemostat, a treatment modality for high pressure bleeding based on super absorbent polymers. Accepted for presentation at the twenty-fifth annual Conference on Shock of the Shock Society. Big Sky, Montana. June 8-11, 2002.

Presentations (three):

Carr, ME. "The BioHemostat – Acute Treatment Modality for High Pressure Bleeding" Presented at the Proceedings of ATACC 2001 – Advanced Technology Applications for Combat Casualty Care. Ft. Walton Beach, FL. September 11, 2001.

Carr, ME. "Development of the BioHemostat Device for Control of High Pressure Bleeding" Presented to the Blood Research Group of the Walter Reed Army Institute of Research in Washington , DC. March 2002.

Carr, ME. "Development of the BioHemostat – A New Treatment for High Pressure Bleeding" Presented at the BIO-Defense & Homeland Security Procurement Conference in Washington, DC. April 30, 2002.

Patent Applications (one):

"BioHemostat – a device for acute treatment of high pressure bleeding." Patent application filed August 2001.

Invention disclosures (two):

"Bio-Hemostat – External Acute Wound Dressing" Filed 31 August 2001.

"Pneumatic Bio-Hemostat – Rapid Pressure Development for Treatment of High Pressure Bleeding" Filed 31 August 2001.

Funding applied for (one):

Pre-proposal submitted to the Navy for development of an external device to simultaneously treat infection, bleeding and pain.

H. **CONCLUSIONS** – Work accomplished to date indicates that the underlying premise of this project is sound. A prototype device has been fabricated which rapidly swells and produces force. The ability to accomplish similar results in blood has been demonstrated at least in principle. Since we are not committed to any particular hemostatic agent, the potential to incorporate (or at least test) a variety of candidate materials is obvious. To this point, the expense of the device remains minimal, and it should be relatively simple to mass-produce a similar product. The potential for utilizing the hydrophilic polymer to not only apply direct pressure but to also serve as a drug delivery system for analgesics and antibiotics (both internally and externally) holds promise not anticipated in the original application (figure 20). The uniqueness of this approach is obvious and to this point the results have been gratifying.

Animal testing which will begin in the near future will be critical to the ultimate goal of moving this potential treatment into human trials. If development continues along its current trajectory one would anticipate that this device should have utility in both compressible and non-compressible bleeding. If this is correct, it will be superior to all currently available treatments, and tourniquets will no longer be required. The treatment paradigm will have shifted from save the patient and then worry about the limb to save the patient and preserve the limb. In the process, the patient and the initial care provider will have a better treatment for the primary cause of death in trauma.

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